Lethbridge iGEM Collegiate 2014 Notebook – CELL CULTURE (September)

NOTE: For detailed protocols regarding starting/passaging/freezing/lipofecting cell cultures and preparing media, see "Cell Culture Protocols" document.

September 4, 2014

- Started three HEK-293 cell culture plates from three aliquots of frozen cell stocks.

September 5, 2014

- Changed media in HEK-293 plate cultures.

September 8, 2014

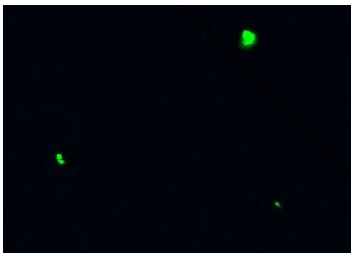
- Split one HEK plate culture into three plate cultures.
- Changed media in one HEK-293 plate culture.
- Lipofected one HEK-293 plate culture with 200ul DNAfectamine (as per manufacturer's protocol) and 10ug of pcDNA3.0-Lamp2B-Clover.

September 9, 2014

- Replaced serum-free media in lipofected and control HEK-293 culture with centrifuged complete media.
- Changed media in recently split HEK-293 plate cultures.
- Received LADMAC and C8-D30 (mouse astrocyte type III) cell lines. Warmed in 37°C water bath for 2min and dripped cell cultures slowly into 9mL complete media. Centrifuged at 125 x g for 7 minutes and resuspended with complete media. Plated (10mL per plate) and incubated at 37°C overnight.

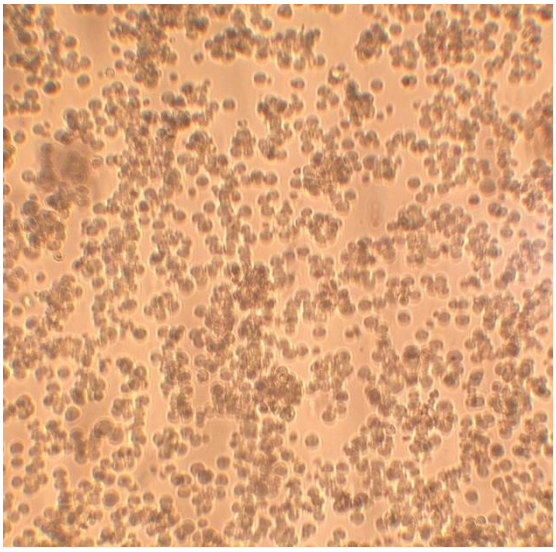
September 10, 2014

- Changed media in C8-D30 plate culture.
- Changed media in HEK-293 plate cultures.
- Removed 2mL of confluent LADMAC culture and added to new plate containing 8mL media.
- Added additional 10mL media to original LADMAC culture.
- Collected lipofected HEK-293 media and control (non-lipofected) HEK-293 media for exosome isolation. Media was filtered and centrifuged (in a SW 41 Ti rotor from Beckman Coulter) at 26,500RPM for 70 minutes at 4°C. Following centrifugation, media was decanted and the pellet was resuspended in PBS buffer.
- Added 10ul drops of PBS-diluted exosome preparations (control and lipofected) to a glass slide. Let dry and then coverslipped with Vectashield. The slide was imaged at 60x with an Olympus FluoView FV1000 confocal scanning laser microscope and green particles were evident in the lipofected samples but not in the control samples (likely attributable to exosome-localized Clover expression).

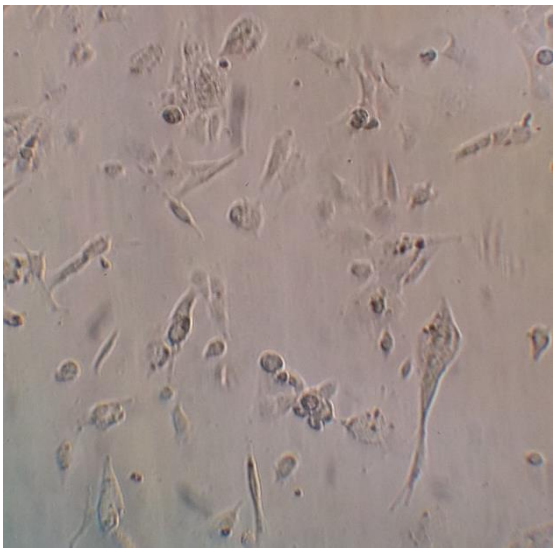


Clumps of exosomes isolated from HEK-293 culture lipofected with pcDNA-Lamp2b-Clover.

September 12, 2014



LADMAC cell culture as seen under a light microscope.



C8-D30 (mouse astrocytes type III) cell culture as seen under a light microscope.

- Changed media in C8-D30 plate culture.
- Changed media in HEK-293 plate cultures.

September 15, 2014

- Froze two HEK-293 plate cultures.
- Split one HEK plate culture into three plate cultures.
- Removed 6mL of confluent LADMAC culture and added 2mL each to three new plates containing 8mL media each.
- Split C8-D30 plate culture into two new plate cultures and one slide culture.

September 16, 2014

- Changed media in C8-D30 plate and slide cultures.
- Changed media in HEK-293 plate cultures.

September 18, 2014

- Changed media in C8-D30 plate cultures.
- Changed media in HEK-293 plate cultures.
- Centrifuged, filtered and froze stocks of LADMAC conditioned media (isolated from original plate). Resuspended cell pellet in freezing buffer and froze 2x aliquots of LADMAC cells.
- Lipofected C8-D30 slide culture with DNAfectamine (as per manufacturer's protocol):
    - 2 chambers: Controls
    - 2 chambers: pcDNA3.0-TEV protease (1ug DNA and 10ul DNAfectamine per chamber)
    - 2 chambers: pcDNA3.0-GFAP-dTomato (1ug DNA and 10ul DNAfectamine per chamber)
    - 2 chambers: pcDNA3.0-NeuroD1 (2ug DNA in one chamber and 1ug DNA in the other plus 10ul DNAfectamine per chamber)

September 19, 2014

- Changed C8-D30 slide culture media to complete media. Added 20ul of Lamp2B-Clover-expressing HEK-293 exosomes to one chamber each of pcDNA3.0-TEV protease lipofected C8-D30, pcDNA3.0-GFAP-dTomato lipofected C8-D30, and control C8-D30. Added 20ul of control non-lipofected HEK-293 exosomes to one chamber each of pcDNA3.0-TEV protease lipofected C8-D30, pcDNA3.0-GFAP-dTomato lipofected C8-D30, and control C8-D30.

September 22, 2014

- Changed media in C8-D30 plate cultures.
- Changed media in HEK-293 plate cultures.
- Did immunocytochemistry on C8-D30 slide culture:
    - Aspirated media and rinsed each chamber with 1x PBS.
    - Fixed with 4% PFA for 20 minutes.
    - Rinsed twice with 1x PBS.

    - Blocked with blocking buffer plus serum for 45 minutes at room temperature.
    - Labeled with rabbit anti-GFAP (1:100 in blocking buffer) for 2hr at room temperature.
    - Rinsed twice with 1x PBS.
    - Labeled all chambers except NeuroD1 with anti-rabbit Alexa Fluor 647 (1:200 in blocking buffer) and NeuroD1 chambers with anti-rabbit Alexa Fluor 488 (1:200 in blocking buffer) for 1hr at room temperature.
    - Rinsed twice with 1x PBS.
    - To NeuroD1 chamber, added anti-NeuN-Cy3 (1:100 in blocking buffer) for 1hr at room temperature.
    - Rinsed twice with 1x PBS.
    - Coverslipped with Vectashield plus DAPI and sealed slide with nailpolish.
- Imaged C8-D30 stained slide on Olympus FluoView FV1000 laser scanning confocal microscope. There was high background fluorescence evident and poor staining (data not shown). Can see some green spots (possibly Lamp2b-Clover exosomes) in cytosol of astrocytes with added exosomes but cannot distinguish from background (may be too dilute; data not shown).

September 23, 2014

- Split one HEK plate culture into three plate cultures.
- Split one C8-D30 plate culture into three slide cultures.
- Lipofected two HEK-293 plate cultures with 200ul DNAfectamine (as per manufacturer's protocol) and 10ug of pcDNA3.0-Lamp2B-Clover each for exosome isolation.

September 24, 2014

- Changed media in C8-D30 cultures.
- Changed media in HEK-293 cultures.

September 25, 2014

- Centrifuged, filtered and froze stocks of LADMAC conditioned media. Resuspended cell pellet and replated in fresh media.
- Collected lipofected HEK-293 media for exosome isolation. Media was filtered and centrifuged (in a SW 41 Ti rotor from Beckman Coulter) at 26,500RPM for 70 minutes at 4°C. Following centrifugation, media was decanted and the pellet was resuspended in PBS buffer.
- To test if lipofection was killing the C8-D30 cultures (or if antibody is causing widespread background fluorescence), lipofected one C8-D30 slide culture with DNAfectamine (as per manufacturer's protocol):
    - 2 chambers: Non-transfected controls
    - 2 chambers: pcDNA3.0-TEV protease (1ug versus 2ug DNA and 10ul DNAfectamine per chamber)
    - 2 chambers: pcDNA3.0-GFAP-dTomato (1ug versus 2ug DNA and 10ul DNAfectamine per chamber)
    - 2 chambers: Transfected controls

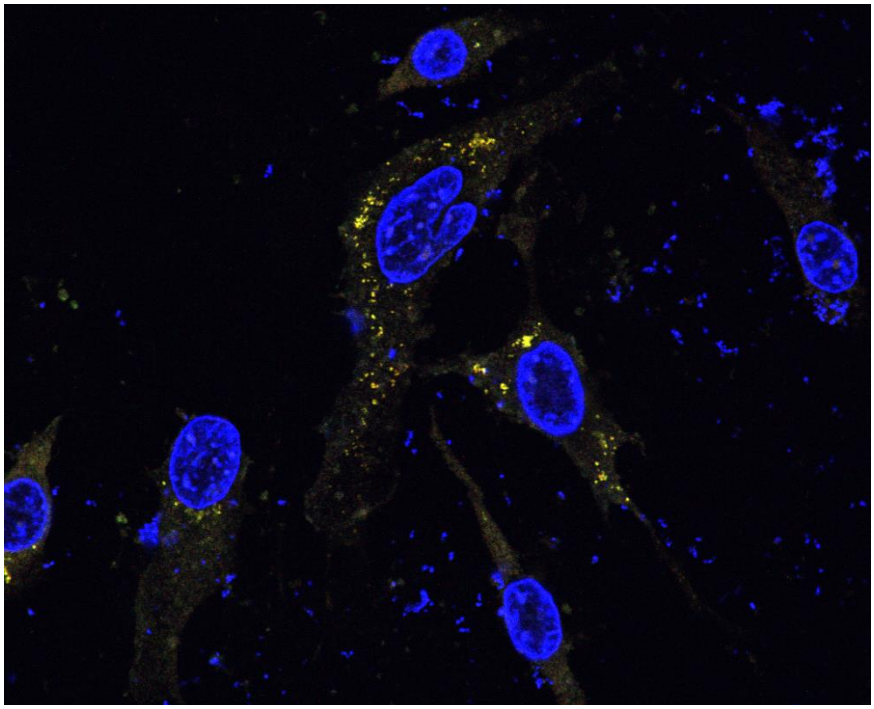
- Lipofected one C8-D30 slide culture with DNAfectamine (as per manufacturer's protocol):
    - 2 chambers: Non-transfected controls
    - 2 chambers: pcDNA3.0-NeuroD1 (1ug DNA and 10ul DNAfectamine per chamber)
    - 2 chambers: pcDNA3.0-NeuroD1 (2ug DNA and 10ul DNAfectamine per chamber)
    - 2 chambers: pcDNA3.0-NeuroD1 (3ug DNA and 10ul DNAfectamine per chamber)

September 26, 2014

- Changed media in C8-D30 lipofected slide cultures to complete media.
- Changed media in C8-D30 cultures.
- Changed media in HEK-293 cultures.

September 29, 2014

- Changed media in C8-D30 cultures.
- Changed media in HEK-293 cultures.
- Did immunocytochemistry on C8-D30 slide culture:
    - Aspirated media and rinsed each chamber with 1x PBS.
    - Fixed with 4% PFA for 20 minutes.
    - Rinsed twice with 1x PBS.
    - Coverslipped with Vectashield plus DAPI and sealed slide with nailpolish.
- Imaged C8-D30 stained slide on Olympus FluoView FV1000 laser scanning confocal microscope. There was autofluorescence (likely due to apoptosis) evident in the absence of antibodies. The lipofection appears to be killing the C8-D30 cultures.



Autofluorescence was evident in all lipofected cell cultures.

September 30, 2014

- Split one C8-D30 plate culture into three plate cultures.
- Received and plated EOC 13.31 (mouse microglia) culture.